Supplementary Materials and Methods

Immunohistochemistry

Immunohistochemistry for ARX, PDX1, ATRX and DAXX was performed manually for 305 primary NF-PanNET using TMAs. Deparaffinized TMA slides were treated with serial xylene treatments and subjected to antigen retrieval using either heated citrate or ethylenediaminetetraacetic acid solution. Blocking of endogenous peroxidase activity was performed prior or after antigen retrieval with 0.6% H₂O₂ (Merck) in methanol for 15 min. Upon treatment for non-specific binding, TMA sections were incubated with primary antibodies for 1 hour at room temperature or overnight at 4°C, followed by secondary antibody (Leica Microsystems or Immunologic) for 30 min and detected with 3,3-diaminobenzidine (Sigma Aldrich or Immunologic) for 10 min. Washing steps were performed with phosphate buffered saline containing 0.1% Tween-20 for at least 5 min. Sections were counterstained with hematoxylin, dehydrated, and mounted.

Fluorescence in situ hybridization (FISH)

Telomere-specific FISH was performed as previously described.1-4 In brief, TMA sections were cut at 4-um, incubated for 30 min at 55/56°C or 10 min at 65°C, washed three times for 5 min in xylene, rinsed in successive 100%, 95%, and 70% ethanol baths, and washed in double-distilled H₂O and, for a subset of cases, 1% Tween before being placed in antigen unmasking solution in a boiling steamer for 20 to 30 min. Next, slides were rinsed in double-distilled H₂O and dehydrated in successive ethanol washes of 70%, 95%, and 100%. Slides were incubated at 72°C for 10 min or 84°C for 5 min with a fluorescently tagged (either Cy3 or Alexa-488) telomeric-C peptide nucleic acid (PNA) probe complementary to the mammalian telomere repeat sequence ([N-
terminus to C-terminus] CCCTAACCCTAACCCTAA) and hybridized overnight in a dark humidified chamber. Following post-hybridization washes, the slides were counterstained with DAPI (Sigma Aldrich) and mounted with prolong anti-fade mounting medium. Finally, slides were analyzed on a fluorescent light microscope.

References:


